The effect of in situ produced dextran on flavour and texture perception of wholegrain sorghum bread

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Yaqin Wang: Methodology, Investigation, Formal analysis, Writing – original draft.

Antonio Trani : Investigation.

Antti Knaapila : Methodology, Supervision, Validation.

Sami Hietala: Supervision, Validation.

Rossana Coda: Supervision.

Kati Katina : Methodology, Supervision.

Ndegwa Henry Maina: Supervision, Funding acquisition.

To be more specific, Yaqin Wang planned the work together with other authors and performed most of the experiments and prepared the manuscript. The dough and bread preparation and analysis were done by Yaqin and supervised by Rossana Coda, Kati Katina, and Ndegwa Maina in the University of Helsinki (Finland). The sensory evaluations were conducted by Yaqin and supervised by Antti Knaapila in the University of Helsinki. The polyphenolic compound analysis was done by Antonio Trani in the International Center for Advanced Mediterranean Agronomic Studies of Bari (Italy). The rheological characterisation of dextran aqueous solutions was performed by Yaqin and supervised by Sami Hietala in the University of Helsinki.

The effect of in situ produced dextran on flavour and texture perception of wholegrain sorghum bread

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Abbreviations:

C*, critical overlap concentration; CMC, carboxymethyl cellulose; CSSB, control sorghum sourdough bread; CSSD, control sorghum sourdough; CWB, control sorghum-wheat bread; CWB, control wheat bread; DSSB, dextran-enriched sorghum sourdough bread; DSSD, dextran-enriched sorghum sourdough; GAE, gallic acid equivalent; GEM, general edible medium; LAB, lactic acid bacteria; M_v, viscosity average molecular weight; TPA, texture profile analysis; TTA, total titratable acidity; UHPLC, ultra high performance liquid chromatography; WG, wholegrain.

Abstract

This study evaluated the effects of dextran produced in situ by Weissella confusa A16 on the flavour and texture perception of wholegrain sorghum bread containing 50% of wheat flour. Descriptive sensory profiling revealed that sorghum sourdough bread containing in situ produced dextran (0.56% bread weight) was more elastic, adhesive, moist, cohesive, soft, flexible, and smooth compared to control sorghum sourdough or native sorghum breads ($p < 0.05$), consistently with the instrumental data. Fermentation significantly increased off-notes such as sour flavour, bitter taste, and aftertaste probably due to the acids production and release of small molecular weight polyphenol compounds (e.g. caffeic acid). The dextran-enriched sorghum sourdough bread, however, showed a significant reduction of flavour intensity perception compared to the control sorghum sourdough bread, despite similar levels of acidification and polyphenols. A trained sensory panel (n =17) was employed to study specifically the masking effect of dextran on sour and bitter notes in model bread systems containing fixed amounts of tastants (acids or caffeine) and varying concentrations of dextran (0.12 0.96% bread weight) above and below the experimentally determined critical overlap concentration C* of dextran (0.43%, w/w). Breads containing higher levels of dextran exhibited a more cohesive, springy and soft texture with significantly less perceived sourness and bitterness intensity. The flavour suppressing seemed to occur at above the critical overlap concentration but remained unaffected at lower concentrations. Dextran produced by W. confusa A16 is a promising texture-enhancing and flavour-masking agent in wholegrain products, which may lead to future innovations in this area.

Keywords

Dextran; Sorghum; Wholegrain; Fermentation; Flavour; Phenolic compounds

1. Introduction

Wholegrain (WG) products that are rich in dietary fibre and other bioactive components have been increasingly considered as a preferred option than refined products (Seal, Jones, & Whitney, 2006). Researches frequently report that consumption of WG products may be associated with reduced risk of major chronic diseases such as cardiovascular disease, obesity and type II diabetes (Ye, Chacko, Chou, Kugizaki, & Liu, 2012). Sorghum (*Sorghum bicolor* L. Moench) is one of the world's most important and oldest known crops, which is commonly consumed as wholegrain (Taylor, Schober, & Bean, 2006). Aside from its nutrition and health benefits, utilisation of sorghum in human diet would also contribute to global food security and sustainable agricultural production. However, even though its significance in human food has been well known/recognized, sorghum remains underutilized in the food production industry. The main limiting factors and barriers are the flavour and texture characteristics of WG sorghum products.

Incorporation of whole sorghum flour above 16% in wheat bread resulted in darker colour, intense bitter taste, lower specific volume and harder crumb, leading to negative consumer responses (Mariera, Owuoche, & Cheserek, 2017). Bitterness is a common sensory concern of wholegrain foods, which is often recognized as off-flavour (Dlouhowski & Gomez-Carneros, 2000). Phenolic compounds, present in the outer layer (pericarp testa) of the grain, are the most important contributor of bitter taste (Kobue-Lekalake, Taylor, & De Kock, 2007). However, phenolic compounds possess high antioxidant activities and are considered beneficial to human health (Awika & Rooney, 2004). Whole sorghum flour also has lower breadmaking quality than wheat flour due to the different technological functionality and types of proteins and the presence of bran fractions. High levels of substitution of wheat with whole sorghum flour induces "dilution" of gluten network and disruption of dough foam structure (Ferrero, 2017). This further results in decreased dough viscoelasticity and gas retentability and consequently smaller volume and firmer crumb of the final bread.

Sourdough fermentation has been one of the most important bioprocessing technique to improve the nutritional value, texture, microbial safety, and shelf-life of WG and refined baked products (Gobbetti, Rizzello, Di Cagno, & De Angelis, 2014; Katina, Heiniö, Autio, & Poutanen, 2006). The positive effects are mainly attributable to the changes in pH and enzymatic activities, which affect the dough structural and nutritional components. On the other hand, the acidification and other biochemical changes during microbial fermentation might generate or enhance undesired flavours in subsequent bread, such as intense sour flavour and bitter taste (Katina, Poutanen, & Karin, 2004; Meignen et al., 2001).

With tailored fermentation, targeted functional metabolites such as texture enhancing dextrans can be produced. Dextrans are natural hydrocolloids produced extracellularly by lactic acid bacteria (LAB) using sucrose as the substrate (Monsan et al., 2001). High molecular weight linear dextrans are the most important in bakery formulations (Lacaze, Wick, & Cappelle, 2007; Rühmkorf et al., 2012). By binding high amount of water, these dextrans are able to improve dough rheological properties, such as dough strength and water absorption capacity, resulting in increased loaf volume, moist mouthfeel, and crumb softness (Wang et al., 2019; Wang et al., 2018; Zannini, Waters, & Arendt, 2014). Dextrans also act as staling inhibitors which decrease amylopectin recrystallization and moisture loss during bread storage, leading to prolonged shelf life (Wang et al., 2019; Zhang et al., 2018). Such properties, along with their production in situ, enable the replacement of commercial hydrocolloids and circumvents the label requirement, conferring a “clean label” status.

Furthermore, the intensive texture modifications induced by dextran might also alter the flavour of the final breads. It is generally understood that the addition of hydrocolloids leads to a decrease in flavour perception. Texture-flavour interactions have been the subject of numerous studies in the last decades (Baines & Morris, 1987; Cook, Hollowood, Linforth, & Taylor, 2002; Costell, Peyrolón, & Duran, 2000; Koliandris, Lee, Ferry, Hill, & Mitchell, 2008), which were carried out

by addition of hydrocolloids (texturing agents) to model or food systems containing taste/aroma compounds. Two classes of systems were studied: a thickened solution or a gel system. For thickened solutions, the masking effect of a hydrocolloid is a modification of the viscosity above its critical overlap concentration (C*) which coincides with a significant decrease in taste/aroma perception (Baines & Morris, 1987; Pangborn, Trabue, & Szczaesniak, 1973). Additionally, the decrease is dependent on the hydrocolloid type and the nature of the taste or aroma compounds. For instance, Mälkki et al. (1993) compared different polymer solutions (oat gum/bran, CMC, and guar gum) presenting similar viscosity and showed that oat had the greatest suppressing effect on sweetness. Troszyńska et al. (2007) studied four hydrocolloids and reported that CMC above C* was the best masker for bitterness and astringency. Moreover, Pangborn et al. (1973) found sour and sweet taste intensities were markedly reduced in cream than solution above C*, whereas saltiness was not changed. In respect of hydrocolloid gels, an inverse relationship between strain at break (or gel strength) and flavour intensity perception was investigated (Koliandris et al., 2008; Lundgren et al., 1986). Nevertheless, rarely have the impact of hydrocolloid addition on flavour perception of baked goods been studied.

This work aimed to study the impact of in situ produced dextran by Weissella confusa A16 on flavour and texture perception of WG sorghum-wheat (ratio 50:50) bread: (1) to profile and quantify the flavour and texture attributes of the bread, by a trained sensory panel; (2) to analyze non-volatile flavour compounds (e.g. sugars, acids, and phenolic compounds) and instrumental texture parameters of the bread, and relate them to the sensory profiles; (3) to further investigate the hypothesis that dextran presence could mask the negative flavour notes of processed wholegrain products. A strategy of using a specific sensory technique, magnitude estimation, was adopted to follow the changes in taste perception in model breads systems containing dextran of varying concentrations, straddling the C* transition. In this way, two taste modalities were studied, sourness and bitterness, by addition of organic acids (lactic and acetic acids) or caffeine in the breads.

2. Materials and Methods

2.1 Materials

The ingredients common to all breads prepared were wheat flour (Helsingin Mylly Oy, Finland; protein 12.5%, fat 2.1%, moisture 13.4%), red sorghum grains (purchased from the local market in Burkina Faso), fresh yeast (Suomen Hiiva Oy, Finland), sucrose (Rainbow, Finland), salt (Meira Oy, Finland), and oil (Bunge Oy, Finland). The wholegrain sorghum flour was obtained by cleaning and milling the sorghum grains with hulls on a laboratory mill (Retsch GmbH, Germany) with a 0.5 mm sieve at a speed of 15,000 rpm (Wang et al., 2019). The flour was stored at −4 °C until use (within 1 month). Catechin (99%), caffeic acid (>99%), quercetin (>99%), luteolin (>97%), apigenin (>99%), citric acid (99.5%), caffeine, lactic acid (85%), acetic acid (99.85%) and vinegar (10% acetic acid) were purchased from Fluka (Buchs, Switzerland), Sigma-Aldrich, and the local market. All other chemicals were of analytical or HPLC grade.

2.2 Strain Growth and Preparation of Sorghum Sourdough

Weissella confusa A16 previously isolated from Massa (a fried sourdough in Burkina Faso) and available at the Department of Food and Nutrition (University of Helsinki) was employed in this experiment. The strain was selected due to its ability to form high molecular weight dextran composed of 97% α-(1 → 6) linear linkages and 3% α-(1 → 6) branch linkages (Wang et al., 2019). The strain was maintained at −80°C in MRS broth supplemented with glycerol (20%, Sigma-Aldrich).

For sourdough preparation, the starter was first propagated in MRS broth (Oxoid, Basingstoke, UK) anaerobically at 30°C for 24 h, and then sub-cultured in general edible medium (GEM) as described in Wang et al. (2018) at 30°C for 24 h. Microbial cells were obtained subsequently from the overnight incubated GEM through centrifugation (10,000 g x 15 min), washed once with sterile sodium phosphate saline buffer (Sigma-Aldrich) and resuspended in distilled water. Milled sorghum

flour, cell culture, and distilled water were mixed to obtain an initial inoculum size of 10^6 cfu/g dough and a dough yield of 240.

Two types of sorghum sourdoughs were prepared as previously described (Table S1 in Supplementary Material) (Wang et al., 2018). Dextran-enriched sorghum sourdough (DSSD) was prepared by replacing 10% (w/w) of the sorghum flour with sucrose to ensure dextran production. Control sorghum sourdough (CSSD) was prepared without sucrose addition. Fermentations were carried out at 25°C for 24 h. LAB cell counts, sourdough viscosity, pH and total titratable acidity (TTA) were determined at 0 h and after 24 h of fermentation (Wang et al., 2018).

2.3 Determination of Sugars and Organic acids

Sugars and dextran in freeze-dried sourdough samples were quantified using a high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) system as previously described (Katina et al., 2009). To extract organic acids, one gram of freeze-dried sample was mixed with 4 mL of Tris-HCl Buffer (50 mM, pH 8.8) with intensive shaking at 4°C for 1 h. After centrifugation (15,000 *g* x 15 min), an equal volume of perchloric acid (5%) was added to the supernatant and allowed to settle overnight at 4°C. The proteins and other polar precipitates were removed by centrifugation and the supernatants were filtered via 0.45 µm filters. The filtrate was injected to high performance liquid chromatography (HPLC) and analyzed according to Weiss et al. (1993). Organic acids were separated by an Agilent Hi-Plex column (Agilent, CA, USA; 300 × 6.5 mm) and the detection was performed by a dual detector system, a refractive index detector (HP 1047A, HP, USA) and an ultraviolet (UV) detector (Waters 717) at 210 nm. The column was run at 40°C using sulfuric acid (10 mM) as the mobile phase with a flow rate of 0.5 mL/min. The sample volume injected was 20 µL using an autosampler (Waters 717 plus). Lactic acid (Sigma-Aldrich) and acetic acid (Merck) were used as standards for quantification.

2.4 Determination of Polyphenolic Compounds

Polyphenolic compounds of the native sorghum flour and lyophilized sorghum sourdoughs were analyzed using ultra high performance liquid chromatography coupled with photodiode array and mass spectrometer in series connected detectors (UHPLC–PDA–MS). Five grams of powdered samples were weighed in 15 mL centrifuge tubes and mixed with 10 mL of methanol:water:formic acid (80:19.9:0.1). The suspensions were shaken for 1 h using an orbital shaker, sonicated for 15 min (Sonorex RK 510 H, US nominal power 160W, Bandelin, Germany) and stored at 8°C overnight to precipitate proteins and polysaccharides. After centrifugation (8000 *g* x 20 min, 8°C), the clear supernatant was filtered using 0.2 µm regenerated cellulose filters and 5 µL of it was injected into the UHPLC system. The analysis was performed using a Dionex Ultimate 3000 RS system (composed by LPG–3400 RS quaternary pump, WPS-3000 TRS autosampler, TCC-3000 RS column oven and PDA), coupled by a HESI–II probe with the LTQ Velos Pro ion trap mass spectrometer (Thermo Fischer Scientific, Rodano, Milan, Italy). The analytical separation of compounds was performed on the Hipersyl Gold aQ column, 1.9 µm particle size, i.d. 2.1 mm 100 mm length (Thermo Fischer) maintained at 30°C. A binary mobile phase was used (A: 0.1% formic acid in water and; B: 0.1% formic acid in acetonitrile) at a constant flow rate of 0.3 mL/min. The gradient program of solvent A was as follows: 0–1 min isocratic 93%; 1–20 min decreased to 40%; 20–25 min isocratic at 40%; then equilibration at the initial conditions for 10 min. The light absorbance was acquired from 220 to 600 nm. The MS conditions were: capillary temperature 330°C; source heater temperature 280°C; nebulizer N_2 gas; sheath gas flow 33 psi; auxiliary gas flow 5 arbitrary units; capillary voltage -2.8 kV; S-Lens RF Level 60%. Data were acquired in negative ionization mode using a data-dependent mode. The data-dependent settings were: full scan from 140 to 650 m/z, activation level 500 counts, isolation width 2 Da, default charge state 1, CID energy 35. All data were acquired and processed using Xcalibur v.2 (Thermo Fischer Scientific). The identification of compounds was achieved by comparing λ_{max}, $[M-H]^-$ and MS/MS fragmentation patterns with literature data (Kang, Price, Ashton, Tapsell, & Johnson, 2016). The

following compounds were identified both using literature data and external standards injection: catechin, caffeic acid, quercetin, luteolin, apigenin. The area percentage of each peak obtained by integrating the 280 nm chromatogram was used to calculate semi-quantitative data for each identified compound. The total amount of polyphenols of the sorghum extracts was determined with Folin Ciocalteu assay accordingly to Wrolstad et al. (2005). The concentration of each identified compound was calculated by multiplying the area percentage with the total polyphenol content and expressed as µg/g of gallic acid equivalent (GAE).

2.5 Bread Making

Four groups of bread were prepared: control wheat bread (CWB), control sorghum-wheat bread (CSWB), control sorghum sourdough bread (CSSB) and dextran-enriched sorghum sourdough bread (DSSB) (Table S1 in Supplementary Material). An additional control bread was prepared using the CSWB recipe with added glucose (0.77% flour weight) and fructose (1.16%) to determine the impact of free sugar accumulation during dextran synthesis on bread textural properties. The sugar content was calculated based on glucose and fructose level detected after 24 h fermentation of dextran-enriched sorghum sourdough. All sorghum containing breads were formulated using wholegrain sorghum and wheat flour in a ratio of 50:50. Sorghum sourdoughs were applied in baking at 59% of the dough weight. The substitution level (50%) of wholegrain sorghum flour was determined to provide 28 g of wholegrains per 100 g of bread. The Dietary Guidelines for Americans (DGA) announced that a wholegrain food must provide at least 8 g of whole grains per 30 g serving (27 g/100 g) (Fernez et al., 2014). In Denmark (DTU 2008) and Sweden (SNF 2007), a food to be labelled as wholegrain must contain at least 50% (dry matter) of wholegrain ingredients.

The water absorption of wheat flour at 500 FU (60.3%) was measured using a Brabender Farinograph (Brabender GmbH & Co.KG, Germany) equipped with a 300 g mixing bowl, according to AACC method 54-21 (AACC 2000). The optimal water addition of sorghum

containing breads (60%) was determined by mixing dough in a Diosna spiral mixer (Dierks & Söhne GmbH, Germany) for 7 min, followed by subjective assessment. Baking tests were performed following a small-scale straight-dough baking process as previously described (Wang et al., 2018) with slight modifications. Briefly, ingredients were kneaded in the Diosna mixer for 3 min at slow speed and 4 min at high speed. Dough temperature after mixing was 26 ± 1°C. The dough was rested for 15 min in a fermentation cabinet (Lillo, Odder, Denmark) at 35°C with relative humidity 75%, scaled into 250 g portions and moulded manually. Afterwards, the dough was placed in aluminium moulds and proofed for 45 min in the fermentation cabinet. The proven doughs were baked at 200°C for 15 min with 15 s steam injection at the beginning. The breads were depanned and allowed to cool for 1 h at room temperature and then stored in plastic bags. Texture Profile Analysis (TPA) of bread crumbs was performed using a texture analyzer (TA, TA-XT2i, Stable Micro Systems Ltd., UK) with a 36-mm diameter aluminum probe and a 5 kg load cell on days 1 and 4 of storage (Wang et al., 2019). The baking loss, specific volume, staling rate (during 4 days of storage), moisture content (on day 1), pH and TTA, residual sugars and acids of the breads were determined as described by Wang et al. (2019).

2.6 Descriptive Sensory Analysis

2.6.1 Panel Selection

The panelists (n = 17) were recruited from staff and graduate students at University of Helsinki: 9 women and 8 men between 20 and 50 years old. Panelists were selected based on their sensory acuity, for instance, their ability to correctly identify and rank the taste of aqueous solutions containing tastants at two concentrations (w/v): citric acid (0.2%, 0.3%) and caffeine (0.05%, 0.07%). Ethical principles applied in sensory research at the department were evaluated and approved by the University of Helsinki Ethical review board in the humanities and social and behavioural sciences (Statement 46/2016). Sensory profiling of the bread samples was performed using the generic descriptive analysis method (Lawless & Heymann, 2010). The evaluation was

carried out at the sensory laboratory of the University of Helsinki with individual booths and following standard sensory practices (Lawless & Heymann, 2010).

2.6.2 Samples

Breads were baked one day before the training and evaluation as described in section 2.5 and stored at room temperature overnight. Prior to the assessments, samples were sliced into 1 cm thickness (with crust and crumb) from the middle of the bread and presented in lidded plastic boxes (minimize moisture loss) marked with random 3-digit codes. Water was provided for palate rinsing.

2.6.3 Panel Training

The panelists had undergone three 2-h sessions of specialized training in analysis of flavour and texture of breads. In the first session, bread samples (CWB, CSWB, CSSB, and DSSB) were presented to panelists to generate the list of descriptors. A specific lexicon of sensory attributes of sandwich breads from the previously published book (U.S. Wheat Associates & Lesaffre, 2017) was provided to the panelists. Assessors were asked to examine the samples individually with respect to the preliminary attributes and discussed with the panel group followed. The suitable descriptors (discriminate among the samples) were selected by consensus, including 8 texture and mouthfeel attributes and 8 flavour attributes (Table 1). In the following session, the reference standards, the methodology of evaluation and evaluation sequence were developed (Table 1). The references selected for inclusion in the subsequent evaluation were: a) the commercial rye (100%) bread named “Uotilan Maalaisruislimppu”, which was characterized by hard/rigid/coarse crumb and strong sour taste; b) caffeine solution (0.05%) with strong bitter taste; c) roasted wheat bread (10% sucrose addition), which had strong roasted flavour (crust) and sweet taste (crumb), and d) regular wheat bread. In the final session, panelists were asked to rate the attributes on continuous visual analogue scales (0 10) with endpoints anchored with verbal definitions (Table 1).

2.6.4 Evaluation Procedures

Two identical evaluations were performed on two independent days (replicates) with two separate sessions per day: one for evaluation of flavour and the other for evaluation of texture. Flavour attributes were evaluated in the morning session where the three categories of sorghum-containing breads (CSWB, CSSB, and DSSB) were examined. CWB was excluded in the flavour session since wheat and sorghum products exhibit different taste profiles and are therefore not comparable. The commercial rye bread, roasted bread, and caffeine solution were served as reference standards. Texture and mouthfeel attributes were evaluated in the afternoon session where the four types of breads (CWB, CSWB, CSSB, and DSSB) were assessed. Commercial rye bread and regular wheat bread were served as standards. The presentation order of the samples was randomized across the panelists and the evaluation sessions. The Fizz Acquisition 2.51 software (Biosystemes, Courternon, France) was used to collect the ratings.

2.7 Preparation of Food Grade Dextran

Dextran was produced by cultivating *W. confusa* A16 in GEM supplemented with 5% sucrose and incubated in anaerobiosis conditions at 30°C for 72 h. Dextran was isolated and purified from the GEM medium according to a previously established method with slight modification (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008). The GEM medium was diluted 1:2 in sterile phosphate saline buffer (PBS, 0.01 M, pH 7.4, Sigma). The suspensions were shaken at a speed of 125 rpm for 10 min at 4°C. Afterwards, the suspensions were centrifuged at 15,000 *g* for 40 min at 4°C using a Beckman Avanti J-25I centrifuge (USA). The supernatants were collected by decanting and the sediments were discarded. Dextran was recovered from the cell-free supernatant by cold ethanol (99.9%) precipitation method. Three volumes of ethanol (4°C) were added to the supernatant and upon steadily stirring dextran was precipitated out of the solution. The dextran precipitate was added to Milli-Q water with constant heating and stirring at 60°C until completely dissolved. Dextran was re-precipitated and re-dissolved in Milli-Q water as mentioned above. This recovery step was repeated four times to remove impurities and the final dextran aqueous

solution was freeze dried. The purity of the isolated dextran was determined by calculating the ratio between the released glucose amount after hydrolysis (10 mg dextran in 2 mL 1 M sulfuric acid hydrolyzed at 100°C for 2 h) and the initial dextran content by HPAEC-PAD.

2.8 Rheological Characterisation of Dextran Aqueous Solutions

Dextran aqueous solutions were prepared at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.5 and 2.0 g/100g. Samples were prepared by weighing the appropriate amount of Milli-Q water into a beaker and heated at 60°C with magnetic stirring. The freeze-dried dextran was carefully added to the side of the vortex with constant stirring at 200 rpm. The solution was heated at 60°C for 2 h and 40°C overnight and allowed to cool down, with continual stirring, at 25°C to ensure adequate hydration of the polymer chains. The flow characteristics of each solution were determined using a DHR2 rheometer (TA Instruments) with a Peltier heated double wall concentric cylinders geometry (inside cup diameter 40 mm, inner cup diameter 44.68 mm, inner cylinder height 55 mm, immersed height 59.5 mm, operating gap 0.5 mm) at 25°C, for a range of shear rates (10-500 s^{-1}